



Wild relatives of wheat: *Aegilops*–*Triticum* accessions disclose differential antioxidative and physiological responses to water stress

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Received: 28 December 2017 / Revised: 14 April 2018 / Accepted: 21 April 2018 / Published online: 25 April 2018
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Abstract

Wild relatives of wheat are an outstanding source of resistance to both abiotic and biotic stresses. In the present study, we evaluated the activity of four antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX)—along with photosynthetic pigments and shoot biomass in 12 *Aegilops*–*Triticum* accessions with different genomic constitutions and two tolerant and sensitive control varieties under well-watered (WW; 90% FC), moderate (MS; 50% FC) and severe (SS; 25% FC) water stress treatments. The analysis of variance for measured traits indicated highly significant effects of the water stress treatments, accessions, and their interactions. The 12 domesticated and wild relatives of wheat exhibited more variability and greater activity in the expression of antioxidative enzymes than cultivated wheats. While domesticated forms of wheat, *T. aestivum* (AABBDD) and *T. durum* (AABB) seem to have a functionally active antioxidant mechanism, other accessions with alien genomes—*Ae. umbellulata* (UU), *Ae. crassa* (MMDD), *Ae. caudata* (CC), *Ae. cylindrica* (DDCC) and *T. boeoticum* (A^bA^b)—respond to water stress by increasing enzymatic antioxidants as the dominant mechanism that contributes to the retention of oxidative balance in the cell. Furthermore, abovementioned accessions with alien genomes had higher photosynthetic pigment contents (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) under water stress than well-watered conditions. Hence, these accessions could be used in future breeding programs to combine beneficial stress-adaptive characters of alien genomes into synthetic hexaploid wheat varieties in the field, even at limited water supply.

Keywords Biochemical traits · Biplot analysis · Photosynthesis · Water stress · Wheat germplasm

Abbreviations

WW Well-watered treatment
MS Moderate water stress
SS Severe water stress
Chl *a* Chlorophyll *a*

Chl *b* Chlorophyll *b*
Chl *T* Total chlorophyll
CAR Total carotenoid
Pro Protein content
SOD Superoxide dismutase
CAT Catalase
APX Ascorbate peroxidase
GPX Guaiacol peroxidase
SWF Shoot fresh weight
SDW Shoot dry weight

Communicated by H Peng.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11738-018-2673-0>) contains supplementary material, which is available to authorized users.

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Introduction

Water deficit is an environmental stress that has dramatic effects on plant growth and agricultural productivity in many parts of the world (Comas et al. 2013), and can simultaneously affect agro-morphological, physiological and physiological changes in plant tissues, which ultimately reduce

yield performance (Cochard et al. 2002). To improve crop productivity in drought-prone regions, the various mechanisms of complex plant responses to water deficit need to be understood. The drought-induced production of reactive oxygen species (ROS)—including singlet oxygen ($^1\text{O}_2$), superoxide (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2)—affects cell membranes, proteins and DNA, leading to cell death (Ruelland et al. 2009; Yadav 2010). Plants exposed to drought have some defense strategies to detoxify ROS and protect themselves from the negative effects of oxidative stress by producing different types of antioxidants. Antioxidants can be divided into two major classes: (1) enzymatic [monodehydroascorbate reductase (MDHAR), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR)], and (2) non-enzymatic [ascorbate acid (AsA), glutathione (GSH), carotenoids and tocopherols], which together alleviate the effects of oxidative stress (Ashraf 2009; Verma et al. 2014). Antioxidants can directly or indirectly induce drought tolerance in wheat. For example, Singh et al. (2012) identified a direct association between sustained yields in tolerant wheat genotypes and better antioxidant activities. In addition, Farooq et al. (2009a) reported that increased levels of antioxidants might improve drought tolerance in wheat by scavenging ROS. Furthermore, the accumulation of soluble phenolic and free proline is a key strategy for reducing stress-induced losses in plants (Farooq et al. 2009a, b). Moreover, increased accumulation of antioxidants in plants under stress conditions will result in ROS scavenging through hydrogen bonding of its hydroxyl groups to phosphate groups of membranes and the polar groups of proteins (Farooq et al. 2017).

The genetic diversity of crop wild relatives (CWR) is ideal for producing new wheat varieties; breeding lines and cultivated varieties have a narrow genetic base for developing drought-tolerant varieties (Pour-Aboughadareh et al. 2017a). The drought tolerance potential of CWR of wheat is being investigated through several drought-adaptive traits including root system architecture (Bektas et al. 2017), cell membrane stability (Suneja et al. 2017), relative water content and stomatal conductance (Pour-Aboughadareh et al. 2017b), water use efficiency (WUE) (Peleg et al. 2005), osmotic adjustment and carbohydrate remobilization (Reynolds et al. 2007), and proline and ABA contents (Kurahashi et al. 2009). Several studies have identified the antioxidant system as a surrogate for breeding selection at the seedling stage (Esfandiari et al. 2007; Osipova et al. 2011; Varga et al. 2012; Arabbeigi et al. 2014; Kong et al. 2014). For instance, Zhang and Kirkham (1994) revealed that *T. compactum* had higher POD and MDA levels and a less efficient antioxidant system than *T. dicoccum*, *T. dicoccoides*, *T. carthilicum*, *T. durum* and *T. monococcum*. In a recent

study, Suneja et al. (2017) investigated accessions of progenitor wheats—*T. dicoccoides* (AABB genome) and *Ae. tauschii* (DD genome)—in terms of the activity of important ROS scavenging enzymes such as CAT, SOD, GR and APX and related them to plant performance under drought stress. The authors stated that these progenitors would be excellent donors for combining beneficial drought-tolerant traits into a new synthetic hexaploid variety to improve wheat for drought-prone environments.

While some wild relatives and main progenitors of wheat respond well to water stress (refs), there is little information on the status of antioxidant activities of wild relatives in response to water stress. Hence, we investigated the degree of resilience in a set of landrace genotypes and wild relatives of wheat in terms of biomass, physiological parameters and ROS scavenging enzymes.

Materials and methods

Plant materials

This study examined 12 accessions of *Triticum* and *Aegilops* species along with two control varieties (*T. aestivum* cv. Sirvan as a tolerant control and *T. aestivum* cv. Darya as a sensitive control). The materials were selected based on the results of a previous study that evaluated 180 accessions under well-watered and severe drought-stressed conditions for key physiological and photosynthesis parameters at the seedling stage (Pour-Aboughadareh et al. 2017b). Detailed information on the genomic constitution and sampling regions of these accessions is in Table 1.

Growth conditions and experimental setup

A pot experiment was conducted in a glass house at the Department of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin, Iran (36°19'21"N, 36°19'21"E) during 2016–2017. After breaking seed dormancy, seeds of each accession were sown in plastic pots filled with 3 kg of dry soil and sand. The soil was sieved before use and the pots filled with a soil:sand mixture in a ratio of 3:1. The experimental design was a factorial randomized complete block design with three replications. All accessions were grown from seed in a glasshouse maintained at an optimal photoperiod (16/8 h light/dark cycle) and growing temperature (25/20 °C day/night). The plants were well-watered every 1–2 days to maintain $90 \pm 5\%$ field capacity. The water stress treatment was initiated at the three-leaf stage of seedling growth. At this time, the pots were subjected to three water regimes: (1) well-watered (WW, FC = $90 \pm 5\%$); (2) moderate water stress (MS, FC = $50 \pm 5\%$) and (3) severe water stress (SS, FC = $25 \pm 5\%$). The FC was determined by the gravimetric method detailed in Souza et al.

Table 1 Characteristics of genomic constitution and sampling locations of the wild relatives of wheat in this study

No.	Genbank code	Species (genome)	Province	Sampling location
1	IUGB-00485	<i>T. aestivum</i> (ABD)	West Azerbaijan	Urmia
2	IUGB-00038	<i>T. durum</i> (AB)	Isfahan	Golpayegan
3	IUGB-01865	<i>T. boeoticum</i> (A ^b)	Kohgiluyeh and Boyer-Ahmad	Shahr-e Kord—Chaharmahal and Bakhtiari Road
4	IUGB-00346	<i>T. urartu</i> (A ^u)	Kurdistan	Saghez–Marivan Road
5	IUGB-00260	<i>Ae. tauschii</i> (D)	Gilan	Astara
6	IUGB-00025	<i>Ae. speltooides</i> (B)	Kermanshah	Qasr-e Shirin
7	IUGB-00966	<i>Ae. umbellulata</i> (U)	Ilam	Dehloran
8	IUGB-00071	<i>Ae. caudata</i> (C)	Lorestan	Andimeshk
9	IUGB-00318	<i>Ae. triuncialis</i> (UC)	Chaharmahal & Bakhtiari	Yasuj–Borujen Road
10	IUGB-01582	<i>Ae. crassa</i> (DM)	Lorestan	Delfan-e Sanjabi
11	IUGB-00172	<i>Ae. cylindrica</i> (DC)	Zanjan	Zanjan
12	IUGB-00387	<i>Ae. neglecta</i> (UM)	Ilam	Manesht koh region
13	Tolerant control	<i>T. aestivum</i> cv. Sirvan	Seed and Plant Improvement Institute (SPII), Karaj, Iran	
14	Sensitive control	<i>T. aestivum</i> cv. Darya	Seed and Plant Improvement Institute (SPII), Karaj, Iran	

IUGB Ilam University Genbank

(2000). Thirty days after the imposition of watering regimes, one seedling was maintained for recording shoot fresh and dry weights, and leaves of other seedlings were harvested and immediately frozen in liquid nitrogen prior to analysis. Shoot fresh and dry weights, some physiological parameters, and antioxidant enzyme activities were measured.

Shoot fresh weight (SFW), shoot dry weight (SDW) and photosynthetic pigments

The aboveground tissues of a single seedling were harvested, weighted for SFW determination, before being oven dried at 70 °C for 72 h and then weighed for SDW determination. Photosynthetic pigments were measured as described by Arnon (1949). Briefly, lyophilized leaves that harvested from other seedlings (100 mg) were incubated with 20 mL of 80% (v/v) acetone. The solution was centrifuged at 12,000g for 10 min. The supernatant was analyzed by spectrophotometry (4802 UV/VIS UNICO) at 480, 510, 663 and 645 nm to obtain the concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *T*), and carotenoid content (CAR) using the following formulas:

$$\text{Chl } a (\mu\text{mol g}^{-1}\text{FW}) : ((12.7 \times \text{Abs } 663) - (2.69 \times \text{Abs } 645)) \times M$$

$$\text{Chl } b (\mu\text{mol g}^{-1}\text{FW}) : ((22.9 \times \text{Abs } 645) - (4.68 \times \text{Abs } 663)) \times M$$

$$\text{Chl } T (\mu\text{mol g}^{-1}\text{FW}) : ((20.2 \times \text{Abs } 645) - (8.02 \times \text{Abs } 663)) \times M$$

$$\text{CAR} (\mu\text{mol g}^{-1}\text{FW}) : ((7.6 \times \text{Abs } 480) - (1.49 \times \text{Abs } 510)) \times M$$

where *M* is the the amount of fresh weight tissue.

Antioxidant assays

Antioxidant enzymes were extracted as described by Kang and Saltveit (2002), with minor modifications. First, 0.5 g of fresh leaf tissue was homogenized at 4 °C in 1 ml of extraction buffer containing 1 mM EDTA, 3 mM MgCl₂, 0.05 M Tris–HCl buffer (pH 7.5), and 1.5% w/v PVP. For APX assay, the extraction buffer contained 0.2 mM ascorbate; the homogenate was centrifuged at 12,000g for 20 min. Finally, the supernatant was used as the crude extract for the four enzyme estimations—SOD, APX, CAT, and GPX.

Superoxide dismutase (SOD)

SOD activity was measured by estimating its ability to inhibit photochemical reduction of nitro-blue-tetrazolium (NBT) using the method of Dhindsa et al. (1981). The 1565 μl reaction mixture contained 50 mM phosphate buffer (pH 7.8), 75 μM NBT, 13 mM methionine, 0.1 mM EDTA, 25 μl riboflavin, and 40 μl enzyme extract. According to the methodology described by Kang and Saltveit (2002), ribo-

flavin was added last, and the tubes placed 30 cm below two

15 W fluorescent lamps and shaken for 15 min. The absorbance of the reaction mixture was read at 560 nm.

Ascorbate peroxidase (APX)

APX activity was analyzed as described by Chen and Asada (1989) with minor modifications. The reaction mixture included of 60 μ l enzyme extract, 0.1 mM EDTA, 1.54 mM hydrogen peroxide, 1.7 ml phosphate buffer (pH 7.0), and 0.5 mM ascorbate. The oxidation of ascorbate was followed by a decline in absorbance at 290 nm.

Catalase (CAT)

CAT activity was determined following the method of Maehly and Chance (1959) by estimating the rate of disappearance of H_2O_2 in absorbance at 240 nm. The reaction mixture contained 250 μ l of 1% H_2O_2 , 50 μ l extracted enzyme and 750 μ l phosphate buffer (pH 7.4).

Guaiacol peroxidase (GPX)

GPX activity was measured as described by Upadhyaya et al. (1985). For this assay, the reaction mixture contained 20 μ l extracted enzyme, 1.25 mM phosphate buffer (pH 6.1), 0.50 ml of 1% H_2O_2 and 0.50 ml of 1% guaiacol. The increase in absorbance at 420 nm was followed for 1 min.

Protein content

According to the method described by Bradford (1976), total protein content was measured using BSA (bovine serum albumin). All enzyme activity was estimated per milligram of protein per minute and expressed as a percentage of the control.

Statistical analysis

Analysis of variance (ANOVA) was done by PROC GLM using SAS software (SAS Institute, Cary, NC, USA). Differences among the means of accessions were tested using Duncan's test. The relative changes caused by water stress on traits were calculated as follows:

$$\text{RC} = \frac{\bar{X}_{\text{ww}} - \bar{X}_{\text{ws}}}{\bar{X}_{\text{ww}}} \times 100,$$

where \bar{X}_{ww} and \bar{X}_{ws} are the means of a trait in a given accession under wellwatered and water stress treatments, respectively (Fayaz and Arzani 2011). To discover relationships between different physiological traits and antioxidant

enzyme activities, a PCA-based biplot was drawn using the XLSTAT package (Addisonsoft XLSTAT, Paris).

Results

Water stress decreased shoot biomass and leaf protein content

Water stress affected shoot fresh weight (SFW), shoot dry weight (SDW) and leaf protein content, with significant differences among accessions. The interaction between stress treatment and accession was significant (Table 2). Water stress reduced SFWs and SDWs in stressed plants by 20.88 and 33.43% in the MS treatment and 32.71 and 43.92% in the SS treatment, respectively (Table 2). The highest SFWs occurred in *T. aestivum*, *T. durum*, *Ae. triuncialis* and the tolerant control variety in the MS treatment, and *Ae. caudata*, *T. aestivum*, *T. durum* and the tolerant control variety in the SS treatment (Fig. 1a). The highest SDWs occurred in the tolerant control variety followed by *T. aestivum*, *Ae. caudata* and *T. boeoticum* in the MS treatment; and *T. aestivum*, *T. durum*, *Ae. cylindrica* and *T. urartu* in the SS treatment (Fig. 1b). Average leaf protein content declined by 17.88% in the MS treatment and 21.65% in the SS treatment. *T. durum* had the highest leaf protein content followed by the tolerant control variety, *Ae. speltoides* and *Ae. umbellulata* in the MS treatment, and *T. urartu*, *T. durum*, *T. boeoticum* and the tolerant control variety in the SS treatment (Fig. 4a).

Water stress changed photosynthetic pigments

The water stress treatments significantly affected Chl *a*, Chl *b* and Chl *T* contents and the amount of total CAR (Table 2). Differences in these pigments (except CAR) were observed among accessions. Furthermore, the interaction between water stress treatment and accession was significant for all traits. These photosynthetic pigments decreased as the water stress became more severe. The average Chl *a* content decreased by 11.38% in the MS treatment, more so in the tolerant control variety, *Ae. umbellulata* and *Ae. triuncialis*, and 42.19% in the SS treatment, more so in *T. aestivum*, *T. boeoticum* and *Ae. umbellulata* (Fig. 2a). Water stress reduced Chl *b* and Chl *T* contents by 21.86 and 16.14% in the MS treatment and 49.49 and 45.50% in the SS treatment, respectively (Table 2). The highest Chl *b* contents occurred in *Ae. caudata*, *Ae. crassa*, *Ae. triuncialis* and *T. durum* in the MS treatment and *T. aestivum*, *T. boeoticum*, *Ae. triuncialis* and *Ae. umbellulata* in the SS treatment (Fig. 2b). The highest Chl *T* contents occurred in *Ae. caudata*, *Ae. crassa*, *Ae. triuncialis* and *T. durum* in the MS treatment and *T. boeoticum*, *T. aestivum*, *Ae. umbellulata* and *Ae. triuncialis* in the SS treatment (Fig. 3a). Water stress reduced average CAR

Table 2 Analysis of variance, mean values and percentage change in biochemical and physiological traits in wheat germplasm

Source of variation	df	Mean square										
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>T</i>	CAR	Pro	SOD	CAT	APX	GPX	SFW	SDW
Replication	2	0.011	0.011	0.001	0.2	60.289	8.047	249	0.005	319.335	0.018	0.002
Water treatment	2	0.704**	0.631**	2.661**	2.14**	1104.15**	6296.95**	0.75**	4.76**	3057.28**	0.19**	0.02**
Accession	13	0.05**	0.03*	0.14**	0.01 ^{ns}	369.67**	1195.75**	0.19**	1.68**	643.18**	0.13**	0.01**
Water treatment × accession	26	0.04**	0.05**	0.16**	0.01*	308.60**	1466.85**	0.26**	1.31**	387.60**	0.03**	0.002 ^{ns}
Error	82	0.013	0.017	0.05	0.005	53.294	167.36	0.083	0.514	127.66	0.016	0.003
Mean for WW treatment		0.59	0.49	1.08	0.41	47.59	45.01	0.23	1.71	29.74	0.41	0.10
Mean for MS treatment		0.52	0.37	0.91	0.26	31.68	36.08	0.42	2.20	34.86	0.32	0.06
Mean for SS treatment		0.34	0.24	0.59	0.26	34.72	69.31	0.43	2.32	46.38	0.27	0.05
Percentage of change due to MS treatment		11.89	24.74	16.14	34.63	33.43	19.83	-81.13	-28.78	-17.22	20.89	33.46
Percentage of change due to SS treatment		42.18	49.48	45.50	34.64	27.04	-53.97	-83.92	-36.05	-55.92	32.71	43.91

Negative numbers indicate value higher than the WW condition

WW well-watered treatment (90% FC), MS moderate water stress (50% FC), SS severe water stress (25% FC), Chl *a* chlorophyll *a* ($\mu\text{mol g}^{-1}$), Chl *b* chlorophyll *b* ($\mu\text{mol g}^{-1}$), Chl *T* total chlorophyll ($\mu\text{mol g}^{-1}$), CAR total carotenoid ($\mu\text{mol g}^{-1}$), Pro protein content (mg g^{-1}), SOD superoxide dismutase (U ml^{-1}), CAT catalase (U ml^{-1}), APX ascorbate peroxidase (U ml^{-1}), GPX guaiacol peroxidase (U ml^{-1}), SFW shoot fresh weight (g plant^{-1}), SDW, shoot dry weight (g plant^{-1})

***Non-significant, significant at 0.05 and 0.01 probability levels, respectively

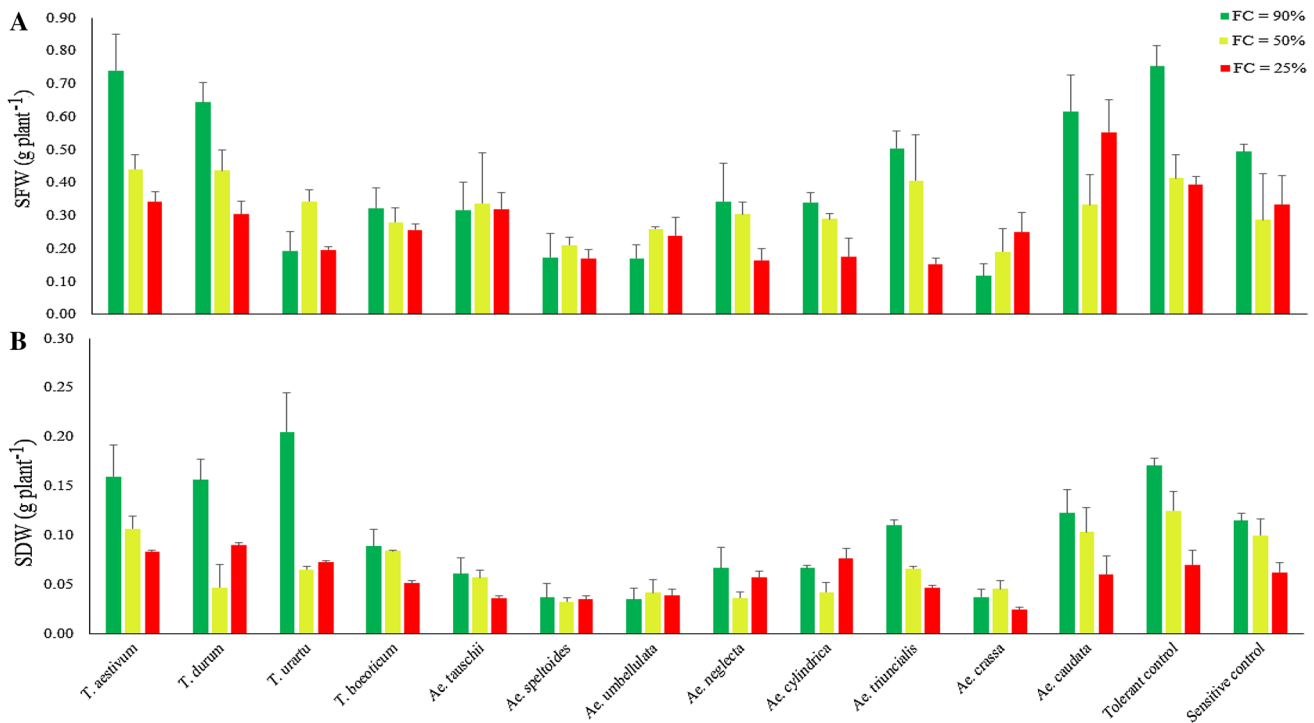


Fig. 1 Shoot fresh weight (g plant⁻¹) (a) and shoot dry weight (g plant⁻¹) (b) in 12 *Aegilops* and *Triticum* accessions and two control varieties (tolerant and sensitive) under well-watered (WW), moderate water stress (MS) and severe water stress (SS) treatments

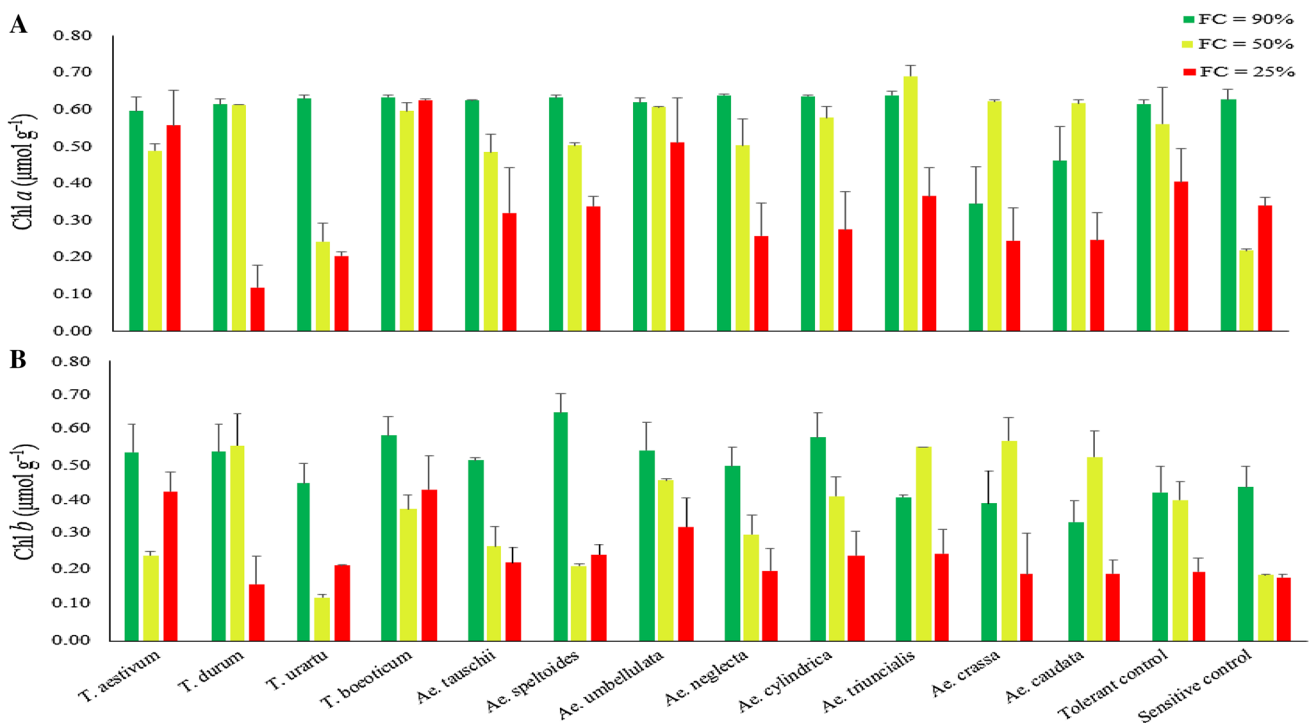


Fig. 2 Chlorophyll a content (µmol g⁻¹ FW) (a) and chlorophyll b content (µmol g⁻¹ FW) (b) in 12 *Aegilops* and *Triticum* accessions and two control varieties (tolerant and sensitive) under well-watered (WW), moderate water stress (MS) and severe water stress (SS) treatments

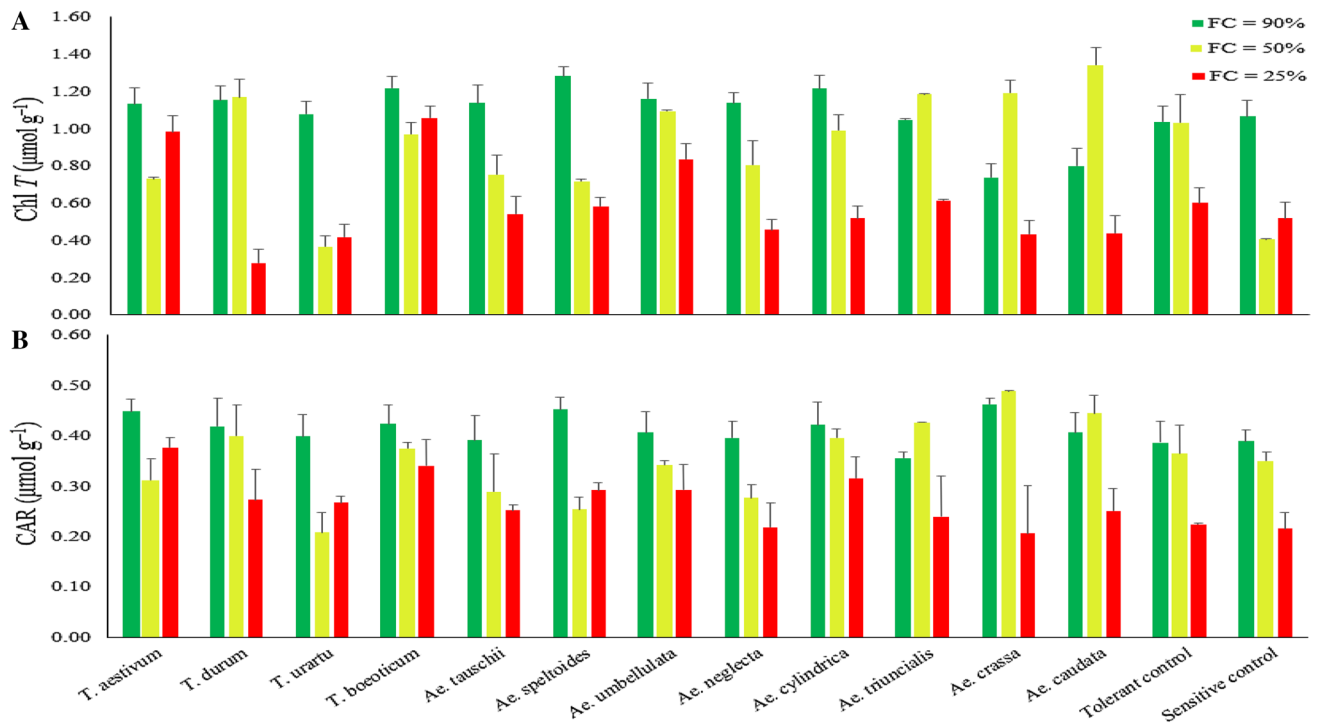


Fig. 3 Total chlorophyll content ($\mu\text{mol g}^{-1}$ FW) (a) and total carotenoid content ($\mu\text{mol g}^{-1}$ FW) (b) in 12 *Aegilops* and *Triticum* accessions and two control varieties (tolerant and sensitive) under well-watered (WW), moderate water stress (MS) and severe water stress (SS) treatments

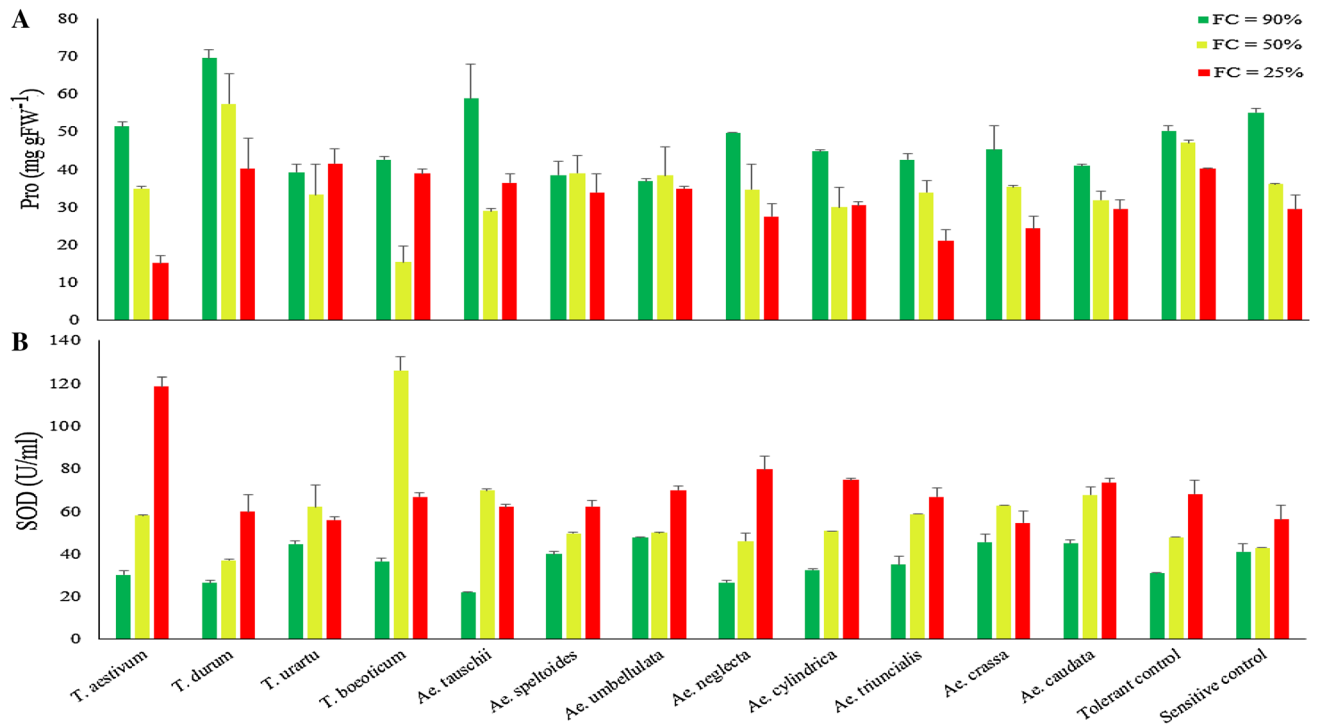


Fig. 4 Leaf protein content ($\mu\text{mol g}^{-1}$ FW) (a) and superoxide dismutase activity (U ml^{-1}) (b) in 12 *Aegilops* and *Triticum* accessions and two control varieties (tolerant and sensitive) under well-watered (WW), moderate water stress (MS) and severe water stress (SS) treatments

values by 15.30 and 34.62% in the MS and SS treatments, respectively (Table 2). The highest CAR values occurred in *Ae. caudata*, *Ae. crassa*, *Ae. triuncialis* and *T. boeoticum* in the MS treatment and *T. aestivum*, *T. boeoticum* and *Ae. cylindrica* in the SS treatment (Fig. 3b).

Water stress improved antioxidant activity

Significant differences occurred in the activity of all antioxidant enzymes between water stress treatments, accessions and their interaction. The water stress treatments significantly increased the antioxidant activities of APX, CAT, SOD and GPX (Table 2). SOD activity increased by 22 and 58% in the MS and SS treatments, respectively. The highest SOD activity occurred in *T. boeoticum*, *Ae. tauschii*, *Ae. crassa* and *Ae. caudata* in the MS treatment and *T. aestivum*, *Ae. neglecta*, *Ae. cylindrica* and *Ae. caudata* in the SS treatment (Fig. 4b). APX activity increased by 32 and 36% in the MS and SS treatments, respectively. The highest APX activity occurred in *Ae. cylindrica*, *Ae. umbellulata*, *Ae. triuncialis* and *T. boeoticum* in the MS treatment and *Ae. crassa*, *Ae. cylindrica*, *Ae. speltoides* and the tolerant control variety in the SS treatment (Fig. 5a). CAT activity increased by 88 and 108% in the MS and SS treatments, respectively. The highest CAT activity occurred in *T. aestivum*, *T. durum*, *T. boeoticum* and *Ae. caudata* in the MS treatment and *Ae. umbellulata*, *Ae. triuncialis*, *Ae. neglecta* and *T. durum* in

the SS treatment (Fig. 5b). GPX activity increased by 22 and 57% in the MS and SS treatments, respectively. The highest GPX activity occurred in *Ae. speltoides*, *Ae. cylindrica*, *T. urartu* and the tolerant control variety in the MS treatment and *Ae. cylindrica* followed by *Ae. speltoides*, *T. urartu* and *Ae. triuncialis* in the SS treatment (Table 2; Fig. 6).

Association among physiological and antioxidant traits

Principal component analysis (PCA) was used to discover relationships between different physiological traits and antioxidant enzyme activities. Associations among traits were considered from the angle between the trait vectors on the biplot. For instance, an acute angle displays a strong positive association, and an obtuse angle indicates a weaker relationship; a 180° angle indicates a negative correlation and a 90° angle indicates no correlation between indices. The results of the PCA-based biplot analyses are shown in Fig. 7a–c. The first two components in Fig. 7a accounted for 56.93% of the total variation in the WW treatment. In this treatment, shoot biomass was strongly correlated with GPX activity and protein content; chlorophyll components (Chl *a*, Chl *b*, Chl *T*) were positively correlated with APX and CAT activities; and CAR had a strong positive correlation with SOD activity. In the MS treatment, the first two components accounted for 48.83% of the total variation (Fig. 7b); all photosynthetic

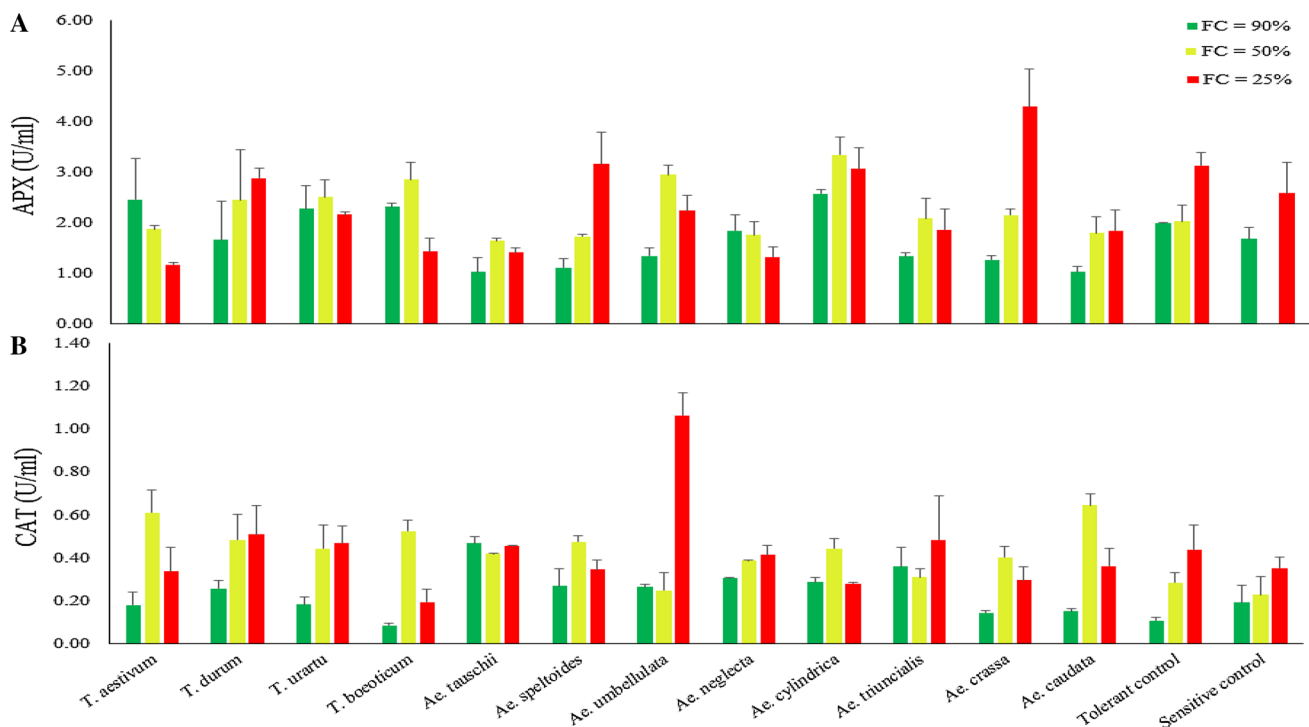


Fig. 5 Ascorbate peroxidase activity (U ml^{-1}) (a) and catalase activity (U ml^{-1}) (b) in 12 *Aegilops* and *Triticum* accessions and two control varieties (tolerant and sensitive) under well-watered (WW), moderate water stress (MS) and severe water stress (SS) treatments

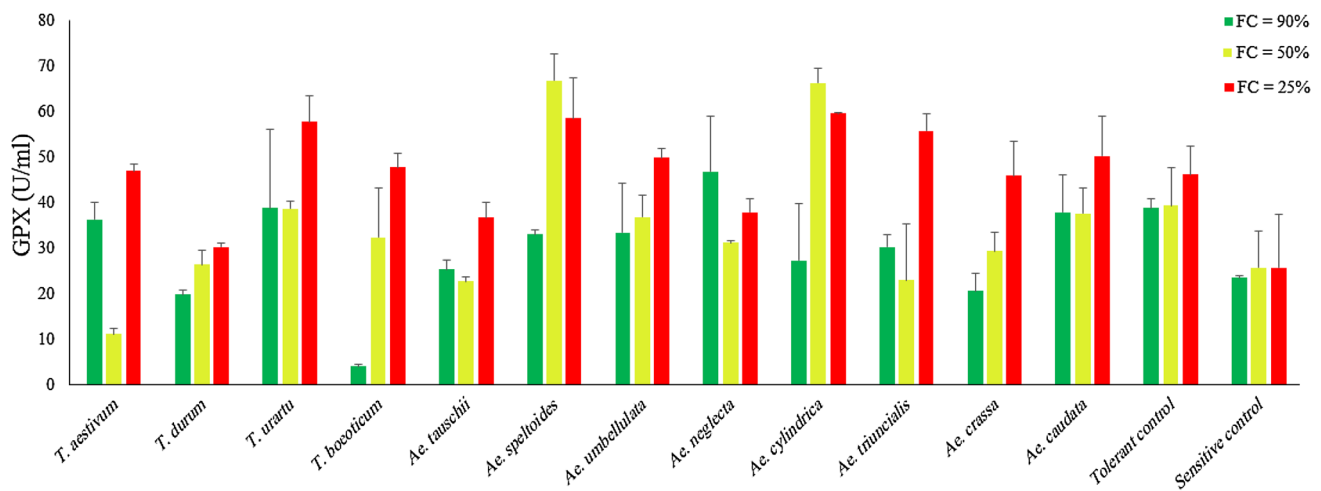


Fig. 6 Guaiacol peroxidase activity (U ml^{-1}) in 12 *Aegilops* and *Triticum* accessions and two control varieties (tolerant and sensitive) under well-watered (WW), moderate water stress (MS) and severe water stress (SS) treatments

pigments were strongly correlated with each other and with CAT activity; CAT was positively correlated with SFW and SDW, and GPX activity was strongly correlated with SOD activity and CAR. In the SS treatment, the correlation patterns among traits differed from the other two treatments; the first two components accounted for 59.32% of the total variation; there was no strong correlation between photosynthetic pigments and shoot biomass; shoot biomass was weakly associated with protein content, and CAT activity had a strong positive association with GPX activity as did protein content and APX activity (Fig. 7c).

Discussion

Plants undergo a variety of changes when exposed to water deficit stress. In such situations, plants not only experience restrictions in growth and development, but also an increase in ROS that presents itself as a secondary stress. Accordingly, plant cells respond to this oxidative stress by actively scavenging excess ROS and preserving antioxidant defense compounds at levels that reflect ambient cellular metabolism (Suneja et al. 2017). Crop wild relatives are increasingly being used in wheat breeding programs as an ideal source of resistance to both abiotic and biotic stresses (Pour-Aboughadareh et al. 2017c). In this study, a representative set of *Aegilops* and *Triticum* accessions were dissected for their photosynthetic pigments and antioxidant potential under water stress at the seedling stage. Our results revealed that water stress treatments decreased shoot fresh and dry biomasses, total protein content and physiological traits in some accessions. In contrast, water stress significantly increased enzymatic activities. Furthermore, the response to water stress differed among accessions (Table 2).

SFW increased in *T. urartu*, *Ae. crassa*, *Ae. tauschii* and *Ae. umbellulata* accessions in the MS and SS treatments, and *Ae. speltoides* in the MS treatment. SDW increased in *Ae. umbellulata* in the MS and SS treatments, *Ae. crassa* in the SS treatment, and *Ae. cylindrica* (DC genome) in the SS treatment (Table S1 and Fig. 1). Rahbarian et al. (2011) reported that increases in SFW and SDW might be related to a drought-adapted root system, such that more root growth enables water and nutrient uptake from deeper in the soil profile. Wild relatives and progenitors of wheat, specifically *Aegilops* species, widely studied for morphological characters and root system architecture to better understand the genetic potential of this germplasm. Understanding the relationships between different drought tolerance adaptive mechanisms and above- and below-ground biomass in wheat germplasm may help in the selection of ideal ideotypes for target conditions such as spring or winter growth habits, and short or long seasons (Bektas et al. 2017). Our findings are consistent with previous studies where drought stress increased seedling shoot and root biomass in wild relatives of wheat and barley (Barati et al. 2015; Pour-Aboughadareh et al. 2017b); this increasing trend during drought stress is a possible drought avoidance mechanism (Guo et al. 2002). The reduction in photosynthetic pigments due to water stress is mainly the result of damage to chloroplasts caused by ROS, such as O_2 and H_2O_2 , leading to lipid peroxidation, and consequently, chlorophyll destruction (Bouchemal et al. 2016). Several studies have reported damage to photosynthetic pigments as a result of water deficit (Kadkhodaie et al. 2014a, b; Bouchemal et al. 2016). Both Chl *a* and Chl *b* are susceptible to drought, which can affect plant performance and grain quality. Because higher chlorophyll contents have been linked to drought tolerance in plants, the selection of individuals based on increased or stable chlorophyll contents

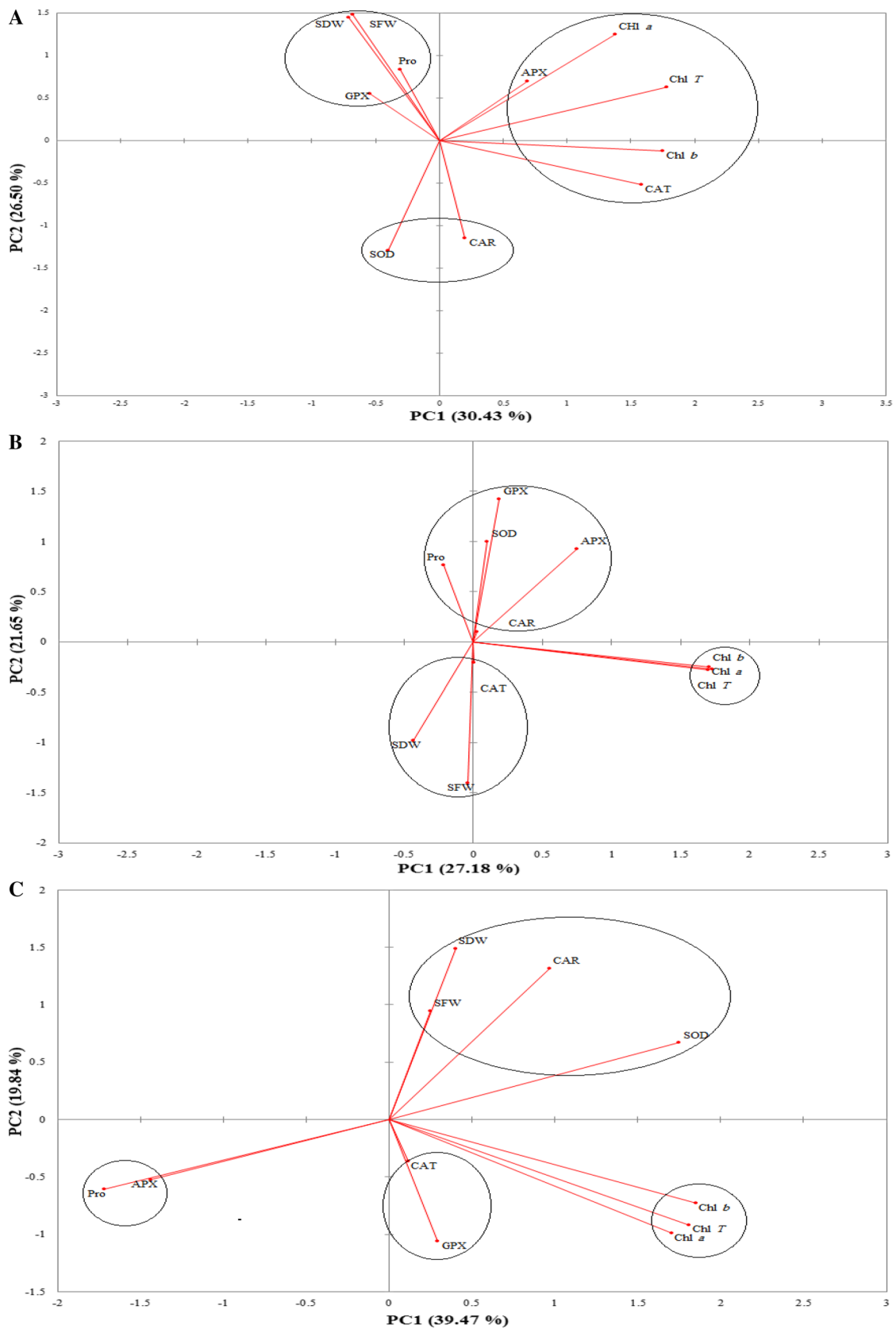


Fig. 7 The biplot display of biochemical and physiological-related traits of domesticated and wild relatives of wheat under **a** well-watered (WW), **b** moderate water stress (MS), and **c** severe water stress (SS) treatments

may inhibit growth and yield reductions under drought stress (Kadkhodaie et al. 2014a).

In the present study, water-stressed accessions of *Ae. triuncialis*, *Ae. caudata* and *Ae. crassa* had higher Chl *a*, Chl *b* and Chl *T* contents than the control, indicating that these accessions may succeed in future breeding programs. The higher chlorophyll contents under water stress in these accessions may be due to the increase in carotenoid content. Carotenoid plays a fundamental role in response to water deficiency conditions and may help plants to tolerate drought stress (Jaleel et al. 2009). The carotenoid content increased in *Ae. triuncialis*, *Ae. caudata* and *Ae. crassa* in the MS treatment and *T. urartu*, *Ae. caudata*, *Ae. umbellulata* and the tolerant control in the SS treatment, relative to the WW control treatment, suggesting that carotenoids play a role as a non-enzymatic antioxidant in the tolerance of wild wheat species to water deficit. The increased carotenoid content in these accessions is likely associated with the absorption of excessive light to avoid photooxidative damage to PSII (Deng et al. 2003). Furthermore, carotenoids are directly involved in reducing chlorophyll, which inhibits the generation of singlet oxygen and oxidative damage (Deng et al. 2003). Similarly, the carotenoid content in the leaves of other crops increased under water deficit stress (Kadkhodaie et al. 2014a, b; Bouchemal et al. 2016; Vieira et al. 2017).

Protein synthesis and accumulation are involved in water stress tolerance. Dehydrins (*Dhn*) and late embryogenesis abundant proteins (*LEA*) are two important protein chaperons that accumulate in response to water deficiency (Ennajeh et al. 2009). However, the extent of accumulation of compatible solutes such as protein and proline varies widely among plant species and genotypes (Kadkhodaie et al. 2014b). In our study, *Ae. umbellulata* and *Ae. speltoides* accessions accumulated more protein in their leaves in the MS treatment, and *T. urartu* accumulated more protein in the SS treatment than the other accessions (Fig. 4a). It appears that increasing protein content during water stress induction is an adaptive mechanism in this germplasm. Antioxidant production increases in response to water deficiency to help plants to cope with drought-induced raised levels of ROS in cells (Sharma et al. 2012). In the present study, the pattern of enzyme activity in the wild relatives of wheat varied under different levels of water stress. Water stress up-regulated the activity of the antioxidative defense system in all accessions and the two control varieties (Table S1). Superoxide dismutase (SOD) plays a central role in the defense against oxidative stress. This biochemical trait is an important parameter for evaluating the water tolerance status of plants (Suneja et al. 2017) and can be used as an indirect selection index for screening drought-resistant plants (Zaefyzadeh et al. 2009). In the current study, the tested accessions varied in their response to water stress. This indicates that different progenitors and wild relatives of wheat have discrete

water stress thresholds. The highest SOD activity occurred in *T. aestivum*, *Ae. tauschii* and *Ae. neglecta* (MS and SS treatments), *Ae. cylindrica* (SS treatment) and *T. boeoticum* (MS treatment), which might protect them from water deficit (Fig. 4b).

Guaiacol peroxidase (GPX) is another major enzyme that has a key role in the defense against stress factors by scavenging H₂O₂ in chloroplasts and biosynthesizing lignin (Gill and Tuteja 2010). In our study, the highest GPX activity occurred in *T. boeoticum*, *Ae. speltoides*, *Ae. triuncialis* and *Ae. crassa* in both water stress treatments (MS and SS) (Table S1 and Fig. 6). Catalase (CAT) turns over rapidly in leaf cells, particularly under stress conditions, and is critical for the scavenging of H₂O₂ formed in the peroxisomes by photorespiration (Noctor et al. 2002). Higher CAT activity will reduce H₂O₂ levels in cells by breaking it down directly to form oxygen and water, and improve CO₂ fixation and membrane stability because several enzymes in the Calvin cycle are very sensitive to H₂O₂ (Esfandiari et al. 2007). In our study, CAT activity increased in *T. aestivum*, *T. boeoticum*, *Ae. crassa* and *Ae. caudata* in the MS treatment and *T. urartu*, *Ae. umbellulata*, *Ae. caudata* and the tolerant control variety in the SS treatment (Table S1 and Fig. 5b). The variation in redox status in the wild relatives of wheat revealed different capabilities of these accessions to deal with oxidative damage by increasing enzyme activity (Pouresmael et al. 2015). In other words, the higher GPX and CAT activities in the accessions listed above revealed a superior capacity for H₂O₂ scavenging compared with the other accessions. Consistent with our results, several studies showed higher levels of GPX and CAT activities in response to water stress than the control (Mafakheri et al. 2010; Pouresmael et al. 2015; Suneja et al. 2017; Vieira et al. 2017). Furthermore, in a study conducted by Sheptovitsky and Brudvig (1996), CAT activity was associated with the photosystem II (PSII) membrane, which may be important to protect chloroplast proteins from damage and stabilize photosynthetic activities. In line with this result, our previous study (Pour-Aboughdareh et al. 2017b) showed that among cultivated varieties and their wild relatives, *Ae. caudata* and *T. urartu* had the highest photosynthetic activity, and were identified as superior candidate species in response to severe drought conditions. Ascorbate peroxidase is an important element of the ascorbate–glutathione (AsA–GSH) cycle, with a significant role in the control of intracellular ROS levels (Sharma et al. 2012). This enzyme is widely distributed in plant cells, and different isoforms are more efficient in scavenging H₂O₂ under stressful environments (Sharma et al. 2012). In our study, the highest APX activity occurred in *Ae. umbellulata*, *Ae. tauschii*, *Ae. crassa* and *Ae. caudata* in the MS treatment and *T. durum*, *Ae. speltoides*, *Ae. crassa* and *Ae. caudata* in the SS treatment (Table S1 and Fig. 5a). In a recent study on the activity of key ROS scavenging enzymes in a set of

wheat progenitors, including diploid and tetraploid accessions, Suneja et al. (2017) found high levels of APX activity in *Ae. tauschii* in response to drought stress.

The PCA analysis identified correlations between enzyme activities and other measured traits. The significant positive association of CAT, SOD, and CAR with shoot biomass under water stress is noteworthy (Fig. 7). Modification of antioxidant enzyme expression due to environmental variability may help to sustain cellular homeostasis and maintain steady-state levels of ROS (Jaleel et al. 2009). Biochemical along with physiological plasticity demonstrated in the form of enzyme induction may contribute to improved performance under drought conditions.

Conclusion and prospects

Although we cannot claim that the differences in each trait are due to species, the main goal of the present study was to disclose the physiological diversity among selected accessions of wild relatives of wheat, and identify some alien genomes for more comprehensive studies. There is also limited knowledge on antioxidant activities and photosynthesis responses among the *Triticeae* tribe; hence, our work addressed interspecies variability. We showed that seedlings of the cultivated genotypes and their wild progenitors differed in their physiological and biochemical traits in response to water stress, and depended on the genomic constitution of the germplasm, thus confirming the existence of a genetically determined tolerance to water deficiency stress among different wild relatives of wheat. Consequently, some wild relatives that have key desirable genes and alleles are more adaptable for improving drought tolerance in cultivated wheat. The wild accessions used in this study exhibited more variability and greater activity in the expression of antioxidative enzymes than the cultivated wheats. While domesticated forms of wheat, *T. aestivum* and *T. durum*, seem to have a functionally active antioxidant mechanism that works constitutively to maintain cellular redox balance, other accessions with alien genomes, such as *Ae. umbellulata* (U genome), *Ae. crassa* (M D), *Ae. caudata* (C genome), *Ae. cylindrica* (DC genome) and *T. boeoticum* (A^b genome), respond to water stress by increasing enzymatic antioxidants as the dominant mechanism contributing to the retention of oxidative balance in the cell. We surmise that species-level differences among these wild relatives might result from different adaptation apparatuses developed over time to cope with unique aspects of environmental factors and local management. Hence, genetic variability in these physiological and biochemical traits could be exploited by crossing wild accessions with modern genotypes to develop new synthetic varieties. These neo-hexaploid forms will combine valuable stress-adaptive features of alien genomes (U, C, A^b,

DM, and DC) into a unique genetic background. Such elite genetic material could be used in chromosome localization, genome mapping and cloning of new stress-responsive genes or alleles for deployment in commercial wheat varieties.

Author contribution statement JA, AP and SF conceived the experiment. AM collected the seeds of the plant material. JA, as the team leader, supervised the process. AP and SF set up the experiment. AP and JA analyzed data and wrote the manuscript. KHMS critically reviewed the manuscript. All authors have read and approved the final manuscript.

Acknowledgements This research was supported by the Iran National Science Foundation (INSF) No. 94010881.

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